



**UNIVERSITI PUTRA MALAYSIA**

**MOLECULAR CHARACTERIZATION OF LISTERIA SPP.  
ISOLATED FROM BEEF, CHICKEN AND FERMENTED  
FISH IN MALAYSIA**

**HAJJAH ENDANG PURWATI RAHAYUNINGSIH**

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**By**

**HAJJAH ENDANG PURWATI RAHAYUNINGSIH**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra  
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Philosophy**

**January 2003**



## DEDICATIONS

To my late mother, Rr. Soejatningsih ,  
My late father, R. Soejono and my mother in law Hajjah Mursiati for their help  
and prayers .....

To my husband, papa Dr. Mursof Fauzi Saladin MD, O & G  
and my daughter Sofrida Prigustina Kurniasari  
for their love, understanding, prayers and patience .....

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy.

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**January 2003**

**Chairman: Dr. Hajjah Zaiton Hassan**

**Faculty : Food Science and Biotechnology**

Modified FDA method was found to give higher recovery of *Listeria* spp. than USDA method for different sources. The results indicated that the imported frozen beef samples from wet market examined were contaminated by seven different *Listeria* spp. namely, *L. monocytogenes*, *L. ivanovii*, *L. seeligeri*, *L. innocua*, *L. welshimeri*, *L. grayi* and *L. murrayi*. However, the use of FDA, USDA and the modified USDA methods may be more beneficial where a limited range of *Listeria* spp. to be recovered (*L. monocytogenes*, *L. ivanovii* and *L. innocua*). *Listeria* spp. was not isolated from any of the 23 samples of imported frozen beef from supermarket and from the five samples of buffalo meat examined.

A total of two hundred and seventy isolates of *Listeria* spp. from different sources were investigated for their susceptibility to 18 antibiotics and were screened for plasmid profiles. Antibiotic susceptibility analysis revealed that all *Listeria* spp. isolates were resistant to two or more antibiotic (MAR 0.11 to 0.66). Majority of the 52 isolates of *L. monocytogenes* displayed resistance to bacitracin, ceftazidime, nalidixic acid, sulfamethazole. However, none were resistant to norfloxacin. Plasmid was detected in 9 (81.8%) of 11 strains of *L. murrayi*, 12 (80%) of 15 strains of *L. grayi*, 29 (55.8%) of 52 strains of *L. monocytogenes*, 8(50%) of 16 strains of *L. denitrificans*, 25 (41.7%) of 60 strains of *L. ivanovii*, 25 (37.9%) of 66 strains of *L. innocua*, 13 (31%) of 42 strains of *L. welshimeri* and 1(12.5%) of 8 strains of *L. seeligeri*. The plasmid sizes ranged from 2.7 to 54 Kb.

In the conjugation study, the donor and the recipient were selected based on the antibiotic resistance pattern of selected *Listeria* spp. isolates from different sources. Seven *L. monocytogenes* strains and one *L. innocua* strain isolated from different sources were selected as donors since they were expected to potentially have an ability to conjugate due to the presence of high molecular weight plasmid DNA (54 Kb). Plasmidless sensitive to antibiotics are selected as recipients. Streptomycin resistance was transferred to *L. monocytogenes* LM65 and LM100 strains at frequencies of

$3.3 \times 10^{-8}$  and  $1.2 \times 10^{-9}$  per input donor cells. A selected kanamycin resistant *L. innocua* strains was found to transfer kanamycin resistant to *L. monocytogenes* (inter-and intra-species transfer).

The use of randomly amplified polymorphic DNA (RAPD) analysis for characterization and differentiation of the isolates of different *Listeria* spp. was examined. The combination of the RAPD-PCR patterns obtained with the three primers (Gen15001, Gen15002 and Gen15010) were able to distinguish all typeable isolates. Base on the dendrograms generated from the RAPD-PCR patterns all *Listeria* spp. isolates could be discriminated and clustered according to their species and food sources. RAPD fingerprinting methods had higher resolution than plasmid profiling and antibiotic resistance patterns and therefore the RAPD fingerprinting methods can be used for epidemiological studies.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia  
sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENCIRIAN SECARA MOLEKULAR KEATAS *LISTERIA* SPESIS YANG  
DIPENCILKAN DARIPADA DAGING LEMBU, DAGING AYAM DAN IKAN  
YANG DITAPAI DI MALAYSIA**

Oleh

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Kaedah pengubahsuaian FDA telah didapati memberikan pulangan yang tinggi untuk spesis *Listeria* berbanding kaedah USDA daripada sumber yang berbeza. Keputusan menunjukkan bahawa sampel daging lembu beku import yang diuji telah dicemarkan oleh tujuh spesis *Listeria* iaitu *L. monocytogenes*, *L. ivanovii*, *L. seeligeri*, *L. innocua*, *L. welshimeri*, *L. grayi* dan *L. murrayi*. Walau bagaimanapun, kegunaan kaedah FDA, USDA dan USDA diubahsuai mungkin lebih menguntungkan dimana spesis *Listeria* yang terhad boleh didapati (*L. monocytogenes*, *L. ivanovii* and *L. innocua*). Spesis *Listeria* tidak dapat dipencilkan daripada mana-mana 23 sampel daging lembu beku import dari supermarket dan 5 sampel daging kerbau yang dikaji.



Sejumlah dua ratus dan tujuh puluh isolat spesies *Listeria* daripada sumber yang telah diperiksa untuk kerektanan kepada 18 jenis antibiotik dan diskriminasi untuk mendapatkan profil DNA (asid deoksiribonuklik) plasmid. Analisis kerektanan antibiotik menunjukkan bahawa kesemua isolat spesies *Listeria* adalah rentang kepada 2 atau lebih antibiotik (MAR 0.11 hingga 0.66). Kebanyakan 52 isolat *L. monocytogenes* mempamerkan kerintangan kepada basitrasin, septazidim, nalidixik asid, sulfamethazole. Walau bagaimanapun, tiada kerintangan kepada norfloxacin. Plasmid telah dikesan di dalam 9 (81.8%) dari 11 strain *L. murrayi*, 12 (80%) dari 15 strain *L. grayi*, 29 (55.8%) dari 52 strain *L. monocytogenes*, 8 (50%) dari 16 strain *L. denitrificans*, 25 (41.7%) dari 60 strain *L. ivanovii*, 25 (37.9%) dari 66 strain *L. innocua*, 13 (31%) dari 42 strain *L. welshimeri*, 1 (12.5%) dari 8 strain *L. seeligeri*, telah menunjukkan kehadiran DNA plasmid. Saiz plasmid berukuran dari 2.7 hingga 54 kb.

Dalam kajian konjugasi, penderma dan penerima telah dipilih berdasarkan corak kerentangan antibiotik spesies *Listeria* yang dipencilkan daripada sumber yang berbeza. Tujuh strain *L. monocytogenes* dan satu *L. innocua* yang dipencilkan daripada sumber berbeza telah dipilih sebagai penderma memandangkan mereka mempunyai potensi dan keupayaan

konjugasi disebabkan kehadiran berat molekul DNA plasmid yang tinggi (54kb) dan penerima mestilah tiada plasmid dan sensitif kepada antibiotik yang dipilih.

Penggunaan analisis DNA polimorfik penggandaan secara rawak (RAPD) untuk pencirian dan pembezaan isolat daripada spesis *Listeria* yang dikaji. Kombinasi corak fingerprinting RAPD yang diperolehi daripada 3 primer (Gen15001, Gen15002 and Gen15010) telah digunakan untuk pembezaan semua isolate. Daripada dendrogram yang digenerasi semua spesis *Listeria* boleh dibezakan dan dikumpulan bergantung kepada spesis dan sumber makanan mereka. Kaedah fingerprinting RAPD adalah lebih sensitif berbanding kepada corak kerintangan antibiotik. Oleh kerana itu, ia digunakan untuk kajian epidemiologi.

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Above all, my praise to Almighty God, Allah SWT. for all His blessing on me and my family.

I certify that an Examination Committee met on 9<sup>th</sup> January 2003 to conduct the final examination of Hajjah Endang Purwati Rahayuningsih on her Doctor of Philosophy thesis entitled "Molecular Characterization of *Listeria* spp. Isolated from Beef, Chicken and Fermented Fish in Malaysia" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examinations Committee are as follows:

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## DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

---

Hajjah Endang Purwati Rahayuningsih

Date:

## TABLE OF CONTENTS

	Page
DEDICATION .....	ii
ABSTRACT .....	iii
ABSTRAK .....	vi
ACKNOWLEDGEMENTS .....	ix
APPROVAL SHEETS .....	xi
DECLARATION FORM .....	xiii
LIST OF TABLES .....	xix
LIST OF FIGURES .....	xx

## CHAPTER

<b>1 GENERAL INTRODUCTION .....</b>	<b>1</b>
<b>2 LITERATURE REVIEW .....</b>	<b>8</b>
2.1 Characteristics and Classification of <i>Listeria</i> spp. ....	8
2.1.1 Taxonomy .....	8
2.1.2 Morphology .....	9
2.1.3 Motility .....	10
2.1.4 Growth .....	10
2.1.4.1 Temperatures .....	10
2.1.4.2 Acidity (pH) .....	11
2.1.4.3 Salt .....	11
2.1.4.4 Water Activity .....	12
2.2 Listeriosis .....	13
2.2.1 Historical Background .....	13
2.2.2 Pathogenesis .....	14
2.2.2.1 Modes of Transmission .....	14
2.2.2.2 Predisposing Factors .....	15
2.2.2.3 Pathogenicity .....	17
2.2.3 Virulence .....	19
2.2.4 Symptoms .....	20
2.2.5 Foodborne Outbreak Caused by <i>L. monocytogenes</i> .....	22
2.2.6 Strategies to Reduce the Problem with <i>L. monocytogenes</i> Infection .....	24





2.3 Detection of <i>L. monocytogenes</i> and <i>Listeria</i> spp. from Food Sources .....	25
2.3.1 Enrichment Procedures of <i>Listeria</i> spp .....	26
2.3.2 Isolation of <i>Listeria</i> spp. ....	29
2.3.3 Identification of <i>Listeria</i> spp. ....	30
2.3.4 Serotype .....	35
2.4 Sensitivity to Antimicrobial Agent .....	36
2.5 Molecular Characterization .....	37
2.5.1 Application of Molecular Techniques in Subtyping of <i>Listeria</i> spp. ....	37
2.5.2 Plasmid DNA Analysis of <i>Listeria</i> spp. ....	38
2.5.3 Bacterial Conjugation .....	42
2.5.4 Polymerase Chain Reaction .....	47
2.5.5 Randomly Amplified Polymorphic DNA of <i>Listeria</i> spp. ...	48
<b>3 METHODOLOGY</b> .....	53
3.1 Isolation, Identification of <i>Listeria</i> spp. and the Ocular Test for the Pathogenicity .....	53
3.1.1 Sample Collection .....	53
3.1.2 Isolation of <i>Listeria</i> spp. ....	54
3.1.3 Identification of <i>Listeria</i> spp. ....	55
3.1.4 Maintenance of Bacterial Strains .....	61
3.1.5 The pathogenicity Tests of <i>L. monocytogenes</i> isolated from Food Using the Ocular Test .....	61
3.2 Antibiotic Susceptibility Testing .....	62
3.3 Plasmid Isolation .....	64
3.3.1 Agarose Gel Electrophoresis .....	66
3.3.2 Staining using Ethidium Bromide .....	67
3.3.3 Visualization of DNA Bands and Photography .....	68
3.3.4 Determinant of Molecular Weight of Plasmid DNA .....	68
3.4 Bacterial Conjugation Studies .....	69
3.5 Randomly Amplified Polymorphic DNA Fingerprinting.....	72
3.5.1 Genomic DNA Extraction for RAPD Fingerprinting.....	72
3.5.2 Spectrophotometric Quantification of DNA .....	73
3.5.3 Standardization of Primer .....	74
3.5.4 RAPD-PCR Amplification .....	75
3.5.5 Analysis of RAPD Fingerprinting Pattern .....	77

<b>4. RESULTS</b>	<b>78</b>
4.1 Comparison of Existing Procedures for the Isolation of <i>L. monocytogenes</i> and Other <i>Listeria</i> spp. from Imported Frozen Beef	78
4.1.1 Prevalence of <i>L. monocytogenes</i> and Other <i>Listeria</i> spp. in Imported Frozen and Local Beef, Chicken and Fermented Fish	81
4.1.2 The Pathogenicity Test of <i>L. monocytogenes</i> Using The Ocular Test	83
4.2 Antibiotic Susceptibility Test and Plasmid profiling of <i>L. monocytogenes</i> and Other <i>Listeria</i> spp. from Different Sources	85
4.2.1 <i>L. monocytogenes</i> strains	85
4.2.2 Antibiotic Susceptibility Test and Plasmid profiling of Other <i>Listeria</i> spp. Isolated from Different Sources	90
4.2.2.1 <i>L. ivanovii</i> strains	96
4.2.2.2 <i>L. seeligeri</i> strains	98
4.2.2.3 <i>L. innocua</i> strains	100
4.2.2.4 <i>L. welshimeri</i> strains	102
4.2.2.5 <i>L. grayi</i> strains	104
4.2.2.6 <i>L. murrayi</i> strains	106
4.2.2.7 <i>L. denitrificans</i> strains	108
4.3 Conjugal Transfer of Plasmid and Antimicrobial Resistance in <i>L. monocytogenes</i> and <i>Listeria</i> spp.	110
4.3.1 The Conjugal Transfer of Streptomycin Resistance of <i>L. monocytogenes</i> Isolated from Imported Frozen Beef	110
4.3.2 The Conjugal Transfer of Tetracycline, Vancomycin and Novobiocin Resistance of <i>L. monocytogenes</i> Isolated from Chicken	111
4.3.3 The Conjugal Transfer of Kanamycin Resistant of <i>L.</i> <i>innocua</i> and <i>L. monocytogenes</i> Isolated from Fermented Fish	114
4.4 Randomly Amplified Polymorphic DNA Fingerprinting	116
4.4.1 Randomly Amplified Polymorphic DNA Analysis for Characterization of <i>Listeria</i> spp.	116
4.4.2 Amplified Polymorphic DNA Analysis for Intra-species Differentiation of <i>Listeria</i> spp. Isolates from Different Sources	137
4.4.2.1 <i>L. monocytogenes</i> strains	137
4.4.2.2 <i>L. ivanovii</i> strains	149
4.4.2.3 <i>L. seeligeri</i> strains	153
4.4.2.4 <i>L. innocua</i> strains	156

4.4.2.5 <i>L. welshimeri</i> strains .....	160
4.4.2.6 <i>L. grayi</i> strains .....	164
4.4.2.7 <i>L. murrayi</i> strains .....	164
4.4.2.8 <i>L. denitrificans</i> strains .....	170
<b>5. DISCUSSION</b> .....	174
5.1 Isolation of <i>L. monocytogenes</i> and Other <i>Listeria</i> spp. Isolated from Different Sources .....	174
5.2 Antibiotic Susceptibility Test and Plasmid Profiling .....	184
5.3 Conjugal Transfer of Plasmid and Antimicrobial Resistance...	190
5.4 Randomly Amplified Polymorphic DNA Fingerprinting .....	196
<b>6. CONCLUSION</b> .....	208
<b>REFERENCES</b> .....	211
<b>APPENDICES</b> .....	232
A1 Photograph of beef samples .....	233
A2 Photographs of plasmid profiles of <i>L. monocytogenes</i> isolated from different sources .....	234
A3 Photographs of plasmid profiles of <i>L. ivanovii</i> isolated from different sources .....	236
A4 Photograph of plasmid profiles of <i>L. seeligeri</i> <i>L. innocua</i> and <i>L. denitrificans</i> isolated from Fermented fish .....	238
A5 Photographs of plasmid profiles of <i>L. innocua</i> isolated from different sources .....	239
A6 Photograph of plasmid profiles of <i>L. welshimeri</i> isolated from different sources .....	240
A7 Photograph of plasmid profiles of <i>L. grayi</i> isolated from different sources .....	241
A8 Photograph of plasmid profiles of <i>L. murrayi</i> isolated from different sources .....	242
B1 Table of antibiotic resistance among <i>Listeria</i> spp. Isolated from imported frozen beef .....	243
B2 Table of antibiotic resistance among <i>Listeria</i> spp. Isolated from local fresh beef .....	244
B3 Table of antibiotic resistance among <i>Listeria</i> spp. Isolated from fermented fish .....	245
C1 Photographs of randomly amplified polymorphic DNA	

	(RAPD-PCR) fingerprinting of <i>L. monocytogenes</i> strains using three primers isolated from different sources .....	246
C2	Photographs of randomly amplified polymorphic DNA (RAPD-PCR) fingerprinting of <i>L. ivanovii</i> strains using three primers isolated from different sources....	254
C3	Photographs of randomly amplified polymorphic DNA (RAPD-PCR) fingerprinting of <i>L. seeligeri</i> strains using three primers isolated from different source....	259
C4	Photographs of randomly amplified polymorphic DNA (RAPD-PCR) fingerprinting of <i>L. innocua</i> strains using three primers isolated from different sources ....	261
C5	Photographs of randomly amplified polymorphic DNA (RAPD-PCR) fingerprinting of <i>L. welshimeri</i> strains using three primers isolated from different sources ....	266
C6	Photographs of randomly amplified polymorphic DNA (RAPD-PCR) fingerprinting of <i>L. grayi</i> strains using three primers isolated from different sources ....	271
C7	Photographs of randomly amplified polymorphic DNA (RAPD-PCR) fingerprinting of <i>L. murrayi</i> strains using three primers isolated from different sources ....	274
C8	Photographs of randomly amplified polymorphic DNA (RAPD-PCR) fingerprinting of <i>L. denitrificans</i> strains using three primers isolated from different sources ....	276
<b>BIODATA OF THE AUTHOR .....</b>		<b>278</b>

## LIST OF TABLES

Table	Page
1 Identification of <i>Listeria</i> spp. ....	34
2 Serotypes of <i>Listeria</i> spp. ....	35
3 FDA, USDA and their modified methods used in this study ....	57
4 Samples tested for isolation and identification of <i>Listeria</i> spp. ....	60
5 List of antibiotics used in the study for antibiotic susceptibility testing ....	63
6 List of sizes in kilobase (Kb) or megadalton (Mda) of <i>Escherichia coli</i> V517 size reference plasmids ....	69
7 List of the oligonucleotide tested for RAPD analysis ....	74
8 The cocktail for the RAPD analysis of <i>Listeria</i> spp. ....	75
9 RAPD amplification of <i>Listeria</i> spp. ....	77
10 <i>Listeria</i> spp. isolated from different enrichment procedures ....	80
11 Incidence of <i>L. monocytogenes</i> and other <i>Listeria</i> spp. in food samples ....	84
12 Antibigram, MAR index and plasmid profiling of <i>L. monocytogenes</i> strains isolated from imported frozen beef, local fresh beef , chicken and fermented fish ....	87
13 Antibigram, MAR index and plasmid profile of Other <i>Listeria</i> spp. isolated from imported frozen beef, local fresh beef , chicken and fermented fish ....	91
14 Characterization of transconjugants among <i>L. monocytogenes</i> isolated from chicken ....	113
15 Genetic transfer of kanamycin resistance among <i>Listeria</i> spp. ....	115
16 Random amplified polymorpgic DNA (RAPD) fingerprinting of the <i>L. monocytogenes</i> and other <i>Listeria</i> isolates using different primers ....	145



## LIST OF FIGURES

Figure	Page
1 Route for transmission of <i>L. monocytogenes</i> to human .....	16
2 Scheme for isolation and detection of <i>Listeria</i> spp. ....	27
3 Plasmids are autonomous circle of DNA .....	41
4 Plasmid protects bacteria against antibiotic .....	41
5 Formation of mating pairs .....	45
6 An F-plasmid with a <i>Tra</i> system .....	45
7 Protocols of USDA and modification USDA for isolation and identification of <i>Listeria</i> spp. from food samples .....	58
8 Protocols of FDA and modification FDA for isolation and identification of <i>Listeria</i> spp. from food samples .....	59
9 Plasmid isolation of <i>Listeria</i> spp. ....	65
10 Genomic DNA isolation .....	76
11 Morphology of <i>L. monocytogenes</i> cells isolated from imported frozen beef using scanning electron microscopy .....	79
12 Percentage of <i>L. monocytogenes</i> strains resistant to antibiotic isolated from different sources .....	89
13 Percentage of <i>L. ivanovii</i> strains resistant to antibiotics isolated from different sources .....	97
14 Percentage of <i>L. seeligeri</i> strains resistant to antibiotics isolated from different sources .....	99
15 Percentage of <i>L. innocua</i> strains resistant to antibiotic isolated from different sources .....	101
16 Percentage of <i>L. welshimeri</i> strains resistant to antibiotics isolated from different sources .....	103
17 Percentage of <i>L. grayi</i> strains resistant to antibiotics isolated from different sources .....	105
18 Percentage of <i>L. murrayi</i> strains resistant to antibiotics isolated from different sources .....	107
19 Percentage of <i>L. denitrificans</i> strains resistant to antibiotics isolated from fermented fish .....	109
20 Agarose gel (0.8%) electrophoresis of plasmid DNA from <i>L. monocytogenes</i> strains isolated from imported frozen beef and their respective transconjugants .....	111
21 Agarose gel (0.8%) electrophoresis of plasmid DNA from <i>L. monocytogenes</i> strains isolated from chicken and their respective transconjugants .....	112

22	Agarose gel (0.8%) electrophoresis of plasmid DNA from <i>Listeria</i> strains isolated from fermented fish and their respective transconjugants .....	114
23	Dendrogram generated from the RAPD patterns when examined with primer Gen15001 of the hemolytic <i>Listeria</i> strains isolated from imported frozen beef .....	119
24	Dendrogram generated from the RAPD patterns when examined with primer Gen15001 of the nonhemolytic strains isolated from imported frozen beef .....	120
25	Dendrogram generated from the RAPD patterns when examined with primer Gen15002 of the hemolytic <i>Listeria</i> strains isolated from imported frozen beef .....	121
26	Dendrogram generated from the RAPD patterns with primer Gen15002 of the nonhemolytic <i>Listeria</i> strains isolated from imported frozen beef .....	122
27	Dendrogram generated from the RAPD patterns with primer Gen15010 of the hemolytic <i>Listeria</i> strains isolated from imported frozen beef .....	123
28	Dendrogram generated from the RAPD patterns with primer Gen15010 of the nonhemolytic <i>Listeria</i> strains isolated from imported frozen beef .....	124
29	Dendrogram generated from the RAPD patterns with primer Gen15001 of the hemolytic <i>Listeria</i> strains isolated from local fresh beef .....	125
30	Dendrogram generated from the RAPD patterns with primer Gen15001 of the nonhemolytic <i>Listeria</i> strains isolated from local fresh beef .....	126
31	Dendrogram generated from the RAPD patterns with primer Gen15002 of the hemolytic <i>Listeria</i> strains isolated from local fresh beef .....	127
32	Dendrogram generated from the RAPD patterns with primer Gen15002 of the nonhemolytic <i>Listeria</i> strains isolated from local fresh beef .....	128
33	Dendrogram generated from the RAPD patterns with primer Gen15010 of the hemolytic <i>Listeria</i> strains isolated from local fresh beef .....	129
34	Dendrogram generated from the RAPD patterns with primer Gen15010 of the nonhemolytic <i>Listeria</i> strains isolated from local fresh beef .....	130
35	Dendrogram generated from the RAPD patterns with primer Gen15001 of the hemolytic <i>Listeria</i> strains isolated from fermented fish .....	131



36	Dendrogram generated from the RAPD patterns with primer Gen15001 of the nonhemolytic <i>Listeria</i> strains isolated from fermented fish .....	132
37	Dendrogram generated from the RAPD patterns with primer Gen15002 of the hemolytic <i>Listeria</i> strains isolated from fermented fish .....	133
38	Dendrogram generated from the RAPD patterns with primer Gen15002 of the nonhemolytic <i>Listeria</i> strains isolated from fermented fish .....	134
39	Dendrogram generated from the RAPD patterns with primer Gen15010 of the hemolytic <i>Listeria</i> strains isolated from fermented fish .....	135
40	Dendrogram generated from the RAPD patterns with primer Gen15010 of the nonhemolytic <i>Listeria</i> strains isolated from fermented fish .....	136
41	Dendrogram generated from the RAPD-PCR analysis using primer Gen15001 (5'-GTGCAATGAG-3') of the <i>L. monocytogenes</i> strains .....	140
42	Dendrogram generated from the RAPD-PCR analysis using primer Gen15002 (5'-CAATGCGTCT-3') of the <i>L. monocytogenes</i> strains .....	141
43	Dendrogram generated from the RAPD-PCR analysis using primer Gen15010 (5'-CCATTTACGC-3') of the <i>L. monocytogenes</i> strains .....	142
44	Dendrogram generated from the RAPD-PCR analysis using primer Gen15001 (5'-GTGCAATGAG-3') of the <i>L. ivanovii</i> strains .....	150
45	Dendrogram generated from the RAPD-PCR analysis using primer Gen15002 (5'-CAATGCGTCT-3') of the <i>L. ivanovii</i> strains .....	151
46	Dendrogram generated from the RAPD-PCR analysis using primer Gen15010 (5'-CCATTTACGC-3') of the <i>L. ivanovii</i> strains .....	152
47	Dendrogram generated from the RAPD-PCR analysis using primer Gen15001 (5'-GTGCAATGAG-3') of the <i>L. seeligeri</i> strains .....	153
48	Dendrogram generated from the RAPD-PCR analysis using primer Gen15002 (5'-CAATGCGTCT-3') of the <i>L. seeligeri</i> strains .....	154
49	Dendrogram generated from the RAPD-PCR analysis using primer Gen15010 (5'-CCATTTACGC-3') of the <i>L. seeligeri</i> strains .....	155



50	Dendrogram generated from the RAPD-PCR analysis using primer Gen15001 (5'-GTGCAATGAG-3') of the <i>L. innocua</i> strains .....	157
51	Dendrogram generated from the RAPD-PCR analysis using primer Gen15002 (5'-CAATGCGTCT-3') of the <i>L. innocua</i> strains .....	158
52	Dendrogram generated from the RAPD-PCR analysis using primer Gen15010 (5'-CCATTTACGC-3') of the <i>L. innocua</i> strains .....	159
53	Dendrogram generated from the RAPD-PCR analysis using primer Gen15001 (5'-GTGCAATGAG-3') of the <i>L. welshimeri</i> strains .....	161
54	Dendrogram generated from the RAPD-PCR analysis using primer Gen15002 (5'-CAATGCGTCT-3') of the <i>L. welshimeri</i> strains .....	162
55	Dendrogram generated from the RAPD-PCR analysis using primer Gen15010 (5'-CCATTTACGC-3') of the <i>L. welshimeri</i> strains .....	163
56	Dendrogram generated from the RAPD-PCR analysis using primer Gen15001 (5'-GTGCAATGAG-3') of the <i>L. grayi</i> strains .....	165
57	Dendrogram generated from the RAPD-PCR analysis using primer Gen15002 (5'-CAATGCGTCT-3') of the <i>L. grayi</i> strains .....	166
58	Dendrogram generated from the RAPD-PCR analysis using primer Gen15010 (5'-CCATTTACGC-3') of the <i>L. grayi</i> strains .....	166
59	Dendrogram generated from the RAPD-PCR analysis using primer Gen15001 (5'-GTGCAATGAG-3') of the <i>L. murrayii</i> strains .....	167
60	Dendrogram generated from the RAPD-PCR analysis using primer Gen15002 (5'-CAATGCGTCT-3') of the <i>L. murrayii</i> strains .....	168
61	Dendrogram generated from the RAPD-PCR analysis using primer Gen15010 (5'-CCATTTACGC-3') of the <i>L. murrayii</i> strains .....	169
62	Dendrogram generated from the RAPD-PCR analysis using primer Gen15001 (5'-GTGCAATGAG-3') of the <i>L. denitrificans</i> strains .....	171

